International Journal of Infectious Diseases 27 (2014) 18–19

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Short Communication

Soil as a source of *Legionella pneumophila* sequence type 47

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ARTICLE INFO

Article history:
Received 25 April 2014
Received in revised form 9 May 2014
Accepted 13 May 2014

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Legionella pneumophila
ST47
Natural soil
Whirlpool
Outbreak
Source investigation

SUMMARY

Legionella pneumophila sequence type (ST) 47 was isolated from soil in a garden. We speculate that this strain was transmitted from soil to the whirlpool in the garden where it caused an outbreak of Legionnaires' disease and Pontiac fever. In the Netherlands, ST47 is frequently isolated from patients, but hardly ever from environmental sources. It is possible that human pathogenic Legionella strains, with ST47 as one of the predominant strains, are transmitted to humans from sources such as natural soil that are currently not targeted in outbreak investigations.

1. Introduction

Legionella pneumophila sequence type (ST) 47 is the sequence type that is most frequently isolated from patients in the Netherlands (41% of *L. pneumophila* serogroup 1 isolates[1]), Belgium (27.9% of *L. pneumophila* serogroup 1 isolates[2]), and the UK (25.7% of *L. pneumophila* isolates[3]). However, in source investigations, this sequence type is rarely found.[1,3] Therefore, it could be questioned whether the sources targeted in source investigations, such as tap water, whirlpools, cooling towers, and potting soil, are the most important sources for the transmission of *L. pneumophila* ST47 to humans.

Since the start of the National Legionella Outbreak Detection Programme in the Netherlands in 2002, *L. pneumophila* ST47 has only been isolated from environmental sources on three occasions, all involving outdoor whirlpools (Table 1[4]). Since all three whirlpools from which the *L. pneumophila* ST47 was isolated were outside in gardens, we speculate that *L. pneumophila* ST47 was introduced into the whirlpools from a reservoir in the open air, possibly soil in the gardens. To test this hypothesis, we sampled soil at two out of the three gardens in which the above mentioned outdoor whirlpools were situated. The soil samples were tested for the presence of *Legionella spp* using an amoebal co-culture procedure.[5] Isolated *L. pneumophila* strains were typed by sequence-based typing.[6,7]

2. Methods

In the garden that was related to the cases in 2013 (garden A), five border soil samples, one soil sample from the lawn, one potting soil sample, and one sample from a compost bin were collected (Table 2). In the garden that was related to a case in 2006 (garden B), eight border soil samples were collected. The soil samples from these two gardens were all collected in September 2013. Samples were taken from the upper 2 cm of soil and stored in sterile bottles at 4 °C until analysis.

For the analysis, approximately 5 g of soil was weighed and 5 ml of Page's amoeba saline solution (PAS)[5] was added. This was vortexed and then incubated at room temperature for 1 h. *Legionella spp* were detected by amoebal co-culture.[5] In brief, *Acanthamoeba castellanii* cells (ATCC 30234; American Type Culture Collection, Rockville, MD, USA) were seeded in 12-well microplates (Corning, NY, USA) at a density of 5×10^5 cells per well in 1 ml of PAS. The incubated soil samples were vortexed vigorously again and for each sample, three wells with amoebae were inoculated with 100 µl sample. After incubation at 32 °C for 3 days, the contents of each well were resuspended by pipetting up

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http://dx.doi.org/10.1016/j.ijid.2014.05.009

Table 1
Overview of source investigations in which *Legionella pneumophila* ST47 was isolated from environmental sources

Year of investigation	Number of cases	Environmental source	Clinical isolate
2013	5 (2 LD, 3 Pontiac fever)	Whirlpool in garden; 200 CFU/l	No
2009	4 (1 LD, 3 Pontiac fever)	Whirlpool in garden; swab sample[4]	No
2006	1 (LD)	Whirlpool in garden; 1 260 000 CFU/l	Yes (ST47)

LD, Legionnaires' disease; CFU, colony-forming units.

Table 2
Results of Legionella detection in samples from garden A

Sample No.	Sample type	Amoebal co-culture[a]	Latex test[b]	Sequence-based typing
1	Border soil	3/3	*L. pneumophila* serogroup 1 (3/3)	ST47 (2/2)
2	Border soil	0/3		
3	Border soil	0/3		
4	Border soil	0/3		
5	Border soil	0/3		
6	Potting soil	3/3	Negative (0/3)	
7	Compost bin	0/3		
8	Lawn soil	3/3	Negative (0/3)	

[a] Number of Legionella-positive wells/number of inoculated wells.
[b] Number of colonies with a positive test result/number of colonies tested.

and down. Subsequently, 100 μl of each well was transferred to a well with freshly seeded amoebae and these were incubated at 32 °C for another 3 days. The contents of each well were 10-fold serially diluted in PAS and plated on buffered charcoal yeast extract (BCYE) plates (Oxoid Ltd, Hampshire, UK). Legionella strains were identified by latex agglutination test (Oxoid Ltd). *L. pneumophila* strains were further genotyped by sequence-based typing.[6,7]

3. Results

Three out of eight samples collected in garden A were positive for *Legionella spp* (Table 2). One border soil sample contained *L. pneumophila* serogroup 1, ST47. The soil sample from the lawn and the potting soil sample contained non-pneumophila Legionella strains that were negative in the latex test. The eight soil samples from garden B were all negative for *Legionella spp*.

4. Discussion

L. pneumophila serogroup 1, ST47 was isolated from natural soil in a garden. *L pneumophila* ST47 had previously been isolated from a whirlpool in that garden during source investigations following a combined Legionnaires' disease and Pontiac fever outbreak in 2013. It is possible that the ST47 strain was transmitted from soil to the whirlpool by wind or by people entering the whirlpool with soil on their feet.

We speculate that direct transmission of ST47 from soil to humans can also occur, for example when handling soil during gardening, causing solitary cases. For many solitary cases, the source of infection remains unknown.

ST47 is hardly ever isolated in source investigations. The isolation of ST47 from natural soil could be an indication that soil is an alternate, yet overlooked source of this human pathogenic strain.

Many studies have described the transmission of human pathogenic *Legionella longbeachae* from potting soil, even though the mode of transmission is not yet clear.[8,9] The transmission of *L. pneumophila* from soil to humans has also been described.[10] Natural soil as a source of pathogenic Legionella bacteria causing human infections warrants further investigation.

Funding: This work was supported by the Ministry of Health, Welfare and Sport (V/151701).

Conflict of interest: All authors have no actual or potential competing interests regarding this article.

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